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THE CONSTITUENTS OF SENNA LEAVES

BY

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CCXIII.—*The Constituents of Senna Leaves.*

By Frank Tutin.

Senna leaves have in the past been the subject of numerous investigations, but until recently little information has been obtained regarding the definite compounds present in them. Most of the investigators have recorded the isolation of "chrysophanic acid" and the "cathartic acid" of Dragendorff—a product to which the purgative action of the drug has been attributed—but which for some time has been recognised as an indefinite mixture of substances.

The most recent investigation of importance on this subject is that by Tschirch and Hiepe (*Arch. Pharm.*, 1900, **238**, 427), where a detailed mention of the previous literature will be found. These investigators described the isolation of the following products: A crystalline substance, $C_{14}H_{10}O_5$; "sennarhamnetin"; "anthraglucosennin"; "senna-emodin"; "sennachrysophanic acid"; "senna*iso*emodin," and amorphous "sennanigrin." No melting point was recorded for the "substance, $C_{14}H_{10}O_5$," and in view of the results of the present investigation it would appear probable that it was a mixture of aloe-emodin and rhein. The latter substance, which has now been isolated from senna, had previously been known to occur only in rhubarb. With regard to the "sennarhamnetin," Tschirch and Hiepe record no analysis, and merely state that the product in question did not melt at 260°. It is now shown, however, that the flavone product present in various specimens of senna leaves consists either of kaempferol (m. p. 274°) or of a mixture of the latter with *iso*rhamnetin (m. p. 302°). The "anthraglucosennin" of the last-mentioned authors would appear to have been a mixture of the glucosides of rhein

and aloe-emodin, the latter compound being the so-called "sennaemodin." "Sennanigrin" is an amorphous, indefinite product, and the occurrence of "sennachrysophanic acid" (chrysophanol) and of "sennaisoemodin"—for which no melting point is given—cannot be confirmed.

With regard to the alleged occurrence of chrysophanic acid in senna, it is stated by Tschirch and Hiepe (*loc. cit.*, p. 435) that the product obtained by them was free from methoxyl, and melted at 171—172°. In subsequent communications, however (Tschirch and Bromberger, *Arch. Pharm.*, 1911, **249**, 222, and Tschirch and Weil, *ibid.*, 1912, **250**, 26), it is emphasised that pure methoxyl-free chrysophanic acid (chrysophanol) melts at 196°. Notwithstanding these differences of melting point, in the former of these papers (*loc. cit.*) it is stated that senna was the first drug in which pure chrysophanol had been observed to occur. It may therefore be noted that the three varieties of senna leaves which have now been examined were found to be devoid of chrysophanol, the only anthraquinone derivatives present being rhein and aloe-emodin.

A summary of the results of the present investigation will be found at the end of this paper.

Experimental.

I. *Tinnevelly Senna Leaves.*

The material employed for this investigation consisted of the best quality of Tinnevelly senna leaves.

A small portion (10 grams) of the ground material was treated with Prollius' fluid, and the resulting extract tested for an alkaloid with the usual reagents, but with a negative result.

Another portion (20 grams) of the ground leaves was successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained:

Petroleum (b. p. 35—50°) extracted	0·89	gram =	4·45	per cent.
Ether	0·25	,,	1·25	,,
Chloroform	0·22	,,	1·10	,,
Ethyl acetate	1·30	,,	6·50	,,
Alcohol	3·18	,,	15·90	,,
Total =	5·84	grams =	29·20	per cent.

For the purpose of a complete examination of the constituents of the leaves, 22·54 kilograms of the dried, ground material were extracted by continuous percolation with hot alcohol. After the removal of the greater part of the solvent, 11·5 kilograms of a viscid, dark green extract were obtained.

Two kilograms of the above-mentioned extract were mixed with

water and distilled in a current of steam, when a quantity (1·5 grams) of a dark-coloured essential oil was obtained, which possessed a strong aromatic odour. There then remained in the distillation flask a dark-coloured aqueous liquid (*A*) and a quantity of a soft, dark green resin (*B*). These products were separated when cold, and the resin washed several times with aqueous ammonium sulphate, the washings being kept separate from the main bulk of the aqueous liquid. This procedure was necessitated by the fact that the resin formed an inseparable emulsion on attempting to wash it with water.

Examination of the Aqueous Liquid (A).

The aqueous liquid, after concentration under diminished pressure, was extracted many times with ether. These ethereal liquids were united, the greater part of the solvent removed, and the residue largely diluted with light petroleum (b. p. 35—50°), when the greater part of the dissolved material was precipitated as a brown solid. On decanting and concentrating the supernatant liquid, and again treating it with light petroleum, further small quantities of a similar solid were obtained, which were added to the first portion. The petroleum liquid was finally evaporated, the residue dissolved in ether, and the ethereal solution shaken successively with aqueous ammonium carbonate, sodium carbonate, and potassium hydroxide.

The ammonium carbonate extract was acidified, and extracted with ether, when a yellow, viscid product was obtained. The latter was digested with warm water and treated with animal charcoal, when the filtrate yielded a small amount of an acid, which separated in colourless needles, melting at 155°, and was identified as salicylic acid.

The sodium carbonate extract yielded only an amorphous, brown powder, but on acidifying the liquid obtained by means of aqueous potassium hydroxide a product was obtained, which was found to consist largely of aloe-emodin. This substance was subsequently obtained in larger amount, and its isolation will be described later.

Isolation of Rhein, $C_{14}H_5O_2(OH)_2 \cdot CO_2H$.

The above-mentioned brown solid which had been precipitated by means of light petroleum was digested with a considerable volume of ether, when a quantity (about 0·5 gram) of a yellowish-brown powder remained undissolved. The latter was collected, and dissolved in pyridine, from which it separated in orange-coloured needles, melting at about 318°. (Found, C = 63·2; H = 2·9. $C_{15}H_8O_6$ requires C = 63·4; H = 2·8 per cent.)

This substance is thus seen to be rhein (4:5-dihydroxyanthraquinone-2-carboxylic acid), a substance which has previously been isolated only from rhubarb. On acetylation it gave diacetylrhein, which separated from acetic anhydride in yellow needles containing solvent of crystallisation, and, after drying at 130°, melted at 258°. The apparently anomalous behaviour which had previously been observed to occur on heating diacetylrhein with xylene (T., 1911, **99**, 952) was found to be due to the loss of acetic anhydride of crystallisation.

The ethereal solution from which the crude rhein had been separated, as above described, was shaken with concentrated aqueous ammonium carbonate, when a yellowish-brown precipitate was produced. The latter was collected, and the aqueous and ethereal layers then separated, when, on acidifying the former, it yielded only a small amount of amorphous, brown material. The above-mentioned precipitate, however, proved to be the ammonium salt of rhein, which, although readily soluble in water, is practically insoluble in concentrated aqueous ammonium carbonate. It yielded rhein, melting at about 318—320°.

Isolation of Kaempferol, $C_{15}H_6O_2(OH)_4$.

The ethereal liquid which had been extracted with aqueous ammonium carbonate, as above described, was shaken with successive portions of dilute aqueous sodium carbonate until the resulting aqueous liquids, the first of which were deep yellow, commenced to acquire a red tint. Each of the alkaline liquids was acidified as soon as it was separated from the ether, and the precipitated solid then collected. On examination, the latter proved to be a mixture of a flavone derivative with some aloe-emodin, and the complete separation of these two substances was found to be possible only by re-dissolving the mixture in ether, and repeating the above-described fractional extraction with dilute aqueous sodium carbonate two or three times, when the aloe-emodin was eventually all left behind in the ether. The flavone derivative, when freed from aloe-emodin, was crystallised by concentrating its solution in slightly diluted alcohol, when it formed small, bright yellow needles, melting at 274°, and this melting point was not altered by further crystallisation. (Found, C = 62·9; H = 3·8. $C_{15}H_{10}O_6$ requires C = 62·9; H = 3·5 per cent.)

The above-described substance, which gave a yellow colour on treatment with alkalis, and dissolved in concentrated sulphuric acid yielding a liquid possessing a strong, blue fluorescence, was identified as kaempferol (1:3:4-trihydroxyflavonol). On acetylation it yielded tetra-acetylkaempferol, which, when crystallised with

ether, ethyl acetate, or alcohol, formed colourless needles, which had a dual melting point, as observed by A. G. Perkin (T., 1902, **81**, 587); thus, fusion first occurs at about 119—120°, the product then gradually resolidifies, and subsequently melts at 183°. (Found, C = 60·3; H = 4·1. $C_{23}H_{18}O_{10}$ requires C = 60·7; H = 4·0 per cent.)

The statement made by Perkin that the dual melting point of tetra-acetylkaempferol is not due to the presence of solvent of crystallisation cannot, however, be confirmed. Specimens crystallised from each of the solvents mentioned, when carefully heated for several hours at 110°, suffered a diminution in weight without undergoing any softening, and then melted sharply at 183°; for example, an air-dried specimen which had been crystallised from ethyl acetate, on drying at 110°, gave the following results:

0·1124 lost 0·0085 of solvent. $C_4H_8O_2 = 8·6$.

$C_{23}H_{18}O_{10},\frac{1}{2}C_4H_8O_2$ requires $C_4H_8O_2 = 8·8$ per cent.

Tetrabenzoylkaempferol.—No benzoyl derivative of kaempferol appears heretofore to have been described. Kaempferol was benzoylated in pyridine solution, the mixture diluted with water, and the precipitated *tetrabenzoylkaempferol* collected. It was very sparingly soluble in most of the usual organic solvents, but separated in tufts of small, colourless needles when its solution in hot xylene was diluted with ethyl acetate. It had a dual melting point, similar to that of the corresponding acetyl derivative, and, in this case also, this behaviour was due to the presence of solvent of crystallisation. Tetrabenzoylkaempferol, after drying at 130°, melts at 205°, then gradually resolidifies, after which it melts at 237—238°. After drying for five hours at 170° it melts at 237—238°, without previous softening:

0·1052 * gave 0·2824 CO_2 and 0·0363 H_2O. C = 73·2; H = 3·8.

$C_{43}H_{26}O_{10}$ requires C = 73·5; H = 3·7 per cent.

No further compound could be isolated from the mother liquors from the above-described kaempferol, but in view of the statement of Tschirch and Hiepe (*Arch. Pharm.*, 1900, **238**, 439) that senna leaves contain "sennarhamnetin," it was deemed desirable further to investigate this point. An additional amount of extract was therefore worked up, and the crude flavone derivative obtained from it was submitted to the action of hydriodic acid in a Zeisel apparatus, but no methyl iodide was evolved. It is thus evident that no rhamnetin, which is a monomethyl ether of quercetin, was present in the Tinnevelly senna leaves. Moreover, another sample of the same variety of leaves yielded a similar product consisting solely of kaempferol.

* Dried at 170°.

Isolation of Aloe-emodin, $C_{14}H_5O_2(OH)_2 \cdot CH_2 \cdot OH$.

The ethereal liquid from which the kaempferol had been removed, as above described, contained a substance which was somewhat slowly extracted by fairly concentrated aqueous sodium carbonate, but quickly removed by dilute potassium hydroxide. It was therefore extracted by means of the latter alkali, after which the ether was found to contain only a small amount of amorphous, neutral material. On acidifying the red, alkaline extracts, a brownish-yellow substance was precipitated, which was collected on a filter. This material was crystallised from ethyl acetate, when it yielded long, lustrous needles of a brownish-orange colour, which melted at about 218°, and proved to be aloe-emodin. (Found, C = 66·5; H = 3·8. Calc., C = 66·7; H = 3·7 per cent.) It was compared with aloe-emodin obtained from aloes and from rhubarb, and found to be identical with both these preparations. It yielded triacetylaloe-emodin, which formed pale yellow needles, melting at 174—174·5°, and tribenzoylaloe-emodin, melting at 232°. In view of the statement of Tschirch and Hiepe (*loc. cit.*, p. 435) that senna leaves contain "sennachrysophanic acid," the mother liquors from the aloe-emodin were carefully investigated for the presence of chrysophanol ("chrysophanic acid"), but with a negative result.

The aqueous liquid from which the above-described compounds had been removed by extraction with ether, as previously mentioned, was acidified with dilute sulphuric acid, when a dark-coloured, amorphous precipitate separated, and was collected. The latter consisted chiefly of resinous material, but it also contained some rhein, which had doubtless been present in the aqueous liquid in the form of a salt. The aqueous liquid, which, after this treatment, was much lighter in colour, was deprived of sulphuric acid by the cautious addition of barium hydroxide, filtered, and then extracted many times with amyl alcohol. The resulting extracts, when washed, and concentrated under diminished pressure to a small bulk, deposited, on cooling, a quantity of a brown, amorphous solid, which was collected. The filtrate from this solid yielded, on evaporation, a smaller amount of a viscid, amorphous product, but it did not appear to contain any tannin, and nothing crystalline could be directly isolated from it. A portion of it was dissolved in water, and heated with dilute sulphuric acid for some time. This resulted in the formation of some brown, resinous material, together with dextrose, but no other definite substance could be isolated. Some amorphous, glucosidic material was therefore present.

The remaining portion of the material readily soluble in cold amyl alcohol was boiled for a minute with dilute aqueous potassium hydroxide, after which it was acidified and extracted with ether. There were then obtained, in addition to brown, amorphous products, small amounts of kaempferol and aloe-emodin.

Isolation of a New Glucoside, Kaempferin, $C_{27}H_{30}O_{16},6H_2O$.

The material which had separated on concentrating the amyl alcohol solution, as above described, was deprived of the latter solvent, dissolved in boiling alcohol, and treated with animal charcoal, when, on cooling the filtrate, yellowish-brown, somewhat gelatinous granules separated. A little water was then added, and the mixture kept in a flask for some five months, alcohol being added from time to time to compensate for the loss by evaporation. At the end of this time yellow crystals commenced to form, and gradually spread throughout the liquid, the granular material at the same time passing into solution. The crystalline solid was then collected, and recrystallised from water, when it quickly separated in yellow needles, which melted and decomposed at 185—195°. This substance had the properties of a glucoside, since, when heated with dilute sulphuric acid, it yielded kaempferol (m. p. 274°) and dextrose. The latter gave *d*-phenylglucosazone, melting at 212°. No glucoside of kaempferol agreeing in properties with the one here described has previously been known, and the name *kaempferin* is therefore proposed for the new compound:

0·1741,* on heating at 155°, lost 0·0277 H_2O. $H_2O=15·8$.

0·1454 † gave 0·2804 CO_2 and 0·0655 H_2O. $C=52·8$; $H=5·0$.

$C_{27}H_{30}O_{16},6H_2O$ requires $H_2O=15·0$ per cent.

$C_{27}H_{30}O_{16}$ requires $C=53·1$; $H=4·9$ per cent.

0·1039 ‡ gave 0·1978 CO_2 and 0·0492 H_2O. $C=51·9$; $H=5·2$.

$C_{27}H_{30}O_{16},H_2O$ requires $C=51·6$; $H=5·1$ per cent.

It thus appears from the above analytical results that kaempferin yields two molecules of dextrose on hydrolysis. It crystallises from water with 6 molecules of the latter, 5 of which are eliminated at 130° and the remaining one at a higher temperature. *Acetyl-* and *benzoyl-kaempferin* were prepared, but both were found to be uncrystallisable.

The original aqueous alcoholic filtrate from the crude kaempferin could not be caused to deposit any further crystalline substance, although it contained a considerable amount of material. With

* Dried in the air for fourteen hours.

† Dried at 155°.

‡ Dried until constant at 130°.

the exception of the uncrystallisable nature of the latter, its properties indicated it to be a mixture of glucosides of anthraquinone derivatives similar to that occurring in rhubarb (T., 1911, **99**, 957). It was therefore hydrolysed by heating for some time with 2·5 per cent. aqueous sulphuric acid, after which the mixture was extracted several times with ether. The resulting ethereal liquid was then examined in a manner similar to that indicated in connexion with the ether extract of the aqueous liquid, when considerable amounts of rhein and aloe-emodin were obtained, together with a little kaempferol. No chrysophanol was present. The acid aqueous liquid, from which these substances had been removed by means of ether, was deprived of sulphuric acid by treatment with barium hydroxide, when the filtered liquid was found to contain an abundance of sugar. It readily yielded *d*-phenylglucosazone, melting at 212°.

It is evident, therefore, that senna leaves contain, in addition to the new glucoside, kaempferin, a quantity of the glucosides of rhein and aloe-emodin, but, as was ascertained in connexion with the investigation of rhubarb (*loc. cit.*), these substances are devoid of purgative action.

The aqueous liquid which had been extracted with amyl alcohol was freed from the latter solvent, concentrated to a small bulk, and then largely diluted with alcohol. On keeping the mixture it deposited a considerable amount of a crystalline solid, mixed with a dark brown, syrupy product. The solid was collected, and separated so far as possible from the syrup, after which it was found possible to obtain it in a colourless condition by repeated crystallisation from dilute alcohol. This product was found to be a mixture of the sodium, potassium, and magnesium salts of an organic acid, the magnesium salt preponderating. It was fractionally crystallised many times from dilute alcohol, but no separation could be effected. No sparingly soluble salt or crystalline derivative of the acid could be prepared, nor could the acid be isolated except in the form of a gummy mass. It was impossible, therefore, to identify it.

Some of the earlier investigators have recorded the presence in senna of calcium malate and tartrate, but the above-described mixture of salts contained neither of these substances.

The original aqueous-alcoholic liquid which had been decanted from the crude mixture of salts, together with the syrupy material separated from the latter, was deprived of alcohol, diluted with water, and then treated with a slight excess of aqueous basic lead acetate. The yellow precipitate thus formed was collected, and

washed, after which it was suspended in water and decomposed by means of hydrogen sulphide. On concentrating the filtered liquid, a brown, viscid product was obtained, from which nothing crystalline could be directly separated. It was therefore boiled for a minute with dilute aqueous potassium hydroxide, acidified, and extracted with ether. There were thus obtained, in addition to dark brown, amorphous products, a little rhein, a considerable amount of kaempferol, and a moderate quantity of aloe-emodin.

The filtrate from the basic lead acetate precipitate was deprived of lead by means of hydrogen sulphide, filtered, and concentrated under diminished pressure to the consistency of a syrup. This material deposited nothing on keeping, but it evidently contained a large amount of sugar, since it readily yielded *d*-phenylglucosazone, melting at 215°.

Examination of the Resin (B).

The resin (*B*), which formed a dark green, viscid mass, amounted to about 290 grams, being thus equivalent to about 7·0 per cent. of the weight of the leaves employed. It was mixed with purified sawdust, and the dried mixture thoroughly extracted in a large Soxhlet apparatus with petroleum (b. p. 35—50°), ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

This extract of the resin, after complete removal of the solvent, formed a dark green, oily mass, and amounted to 241 grams. It was digested with a large volume of ether, and the mixture cooled and filtered. A quantity (about 3 grams) of a green, sparingly soluble solid was thus obtained, which, after being twice distilled under diminished pressure, and crystallised several times from ethyl acetate, proved to be myricyl alcohol, a larger amount of which was subsequently isolated.

The ethereal solution from which the crude myricyl alcohol had been removed was washed with water, when a dark-coloured, aqueous liquid was obtained. The latter was found to consist of a colloidal solution of a resin, and on treatment with an aqueous solution of an inorganic salt or mineral acid it yielded a dark, resinous precipitate. Nothing definite, however, could be isolated from the latter. The washed ethereal liquid was then shaken successively with aqueous ammonium carbonate, potassium carbonate, and potassium hydroxide. Much difficulty was experienced in separating the resulting aqueous liquids owing to the formation of emulsions, which were found to be caused by the separation

of further amounts of myricyl alcohol, contaminated with much chlorophyll. The dark-coloured alkaline liquid obtained by extraction with ammonium carbonate was acidified, re-extracted with ether, and the ethereal solution fractionally extracted with aqueous ammonium carbonate. Rhein was then obtained, together with some dark-coloured, amorphous products. The alkaline liquid obtained by means of aqueous potassium carbonate, when treated in an analogous manner, yielded chiefly amorphous products, together with small amounts of kaempferol and aloe-emodin, whilst the potassium hydroxide extract yielded considerable amounts of the last-mentioned anthraquinone derivative, but no chrysophanol.

Isolation of a Phytosterolin, $C_{33}H_{56}O_6$.

The ethereal liquid which had been extracted with aqueous alkalis, as above described, was washed with water, when a considerable amount of dark green material was removed. The washings were acidified, and extracted with ether, and the sparingly soluble solid which had separated was then collected on a filter and washed with ether. This solid was thoroughly examined, but appeared to consist only of chlorophyll and resinous matter. The ethereal filtrate was then extracted with fairly concentrated aqueous potassium hydroxide, when small amounts of rhein and aloe-emodin were obtained. After this treatment it was washed with water, which removed a large quantity of a dark green product, whilst some neutral substance which had been occluded in the material removed by the first washing with water, described above, remained dissolved in the ether, and was added to the main bulk of the neutral constituents of the petroleum extract. The aqueous washings were acidified, and extracted with ether, when some sparingly soluble solid which separated in the form of an emulsion was collected, and well washed with ether. The solid was dried, and extracted in a Soxhlet apparatus for a long time with ethyl acetate, after which the boiling liquid was separated by filtration from some sparingly soluble solid. In this way a large amount of chlorophyll and resinous material was removed. The sparingly soluble solid separated from dilute pyridine as a black, amorphous powder, but gave a colour reaction, indicating that it contained a phytosterolin. It was therefore acetylated by heating with acetic anhydride in the presence of pyridine, when the resulting acetyl derivative crystallised from alcohol in dark-coloured leaflets. This material, after treatment with animal charcoal and repeated crystallisation from alcohol, was finally obtained in colourless, glistening leaflets, melting at 163°.

On hydrolysis with potassium hydroxide this acetyl derivative

yielded a substance which crystallised from dilute pyridine in small tufts of colourless, microscopic needles, melting at about 290°:

0·0909 gave 0·2403 CO_2 and 0·0854 H_2O. C=72·1; H=10·4.
$C_{33}H_{56}O_6$ requires C=72·3; H=10·2 per cent.

This substance was thus identified as a phytosterolin (phytosterol glucoside).

The acetyl derivative, on analysis, gave the following result:

0·1076 gave 0·2727 CO_2 and 0·0879 H_2O. C=68·9; H=9·2.
$C_{33}H_{52}O_6(CO\cdot CH_3)_4$ requires C=68·7; H=8·9 per cent.

The original ethereal filtrate from the crude phytosterolin was evaporated, and the very dark green, oily residue dissolved in methyl alcohol, and esterified by means of sulphuric acid. The mixture was then cooled, and filtered, which removed some resin and a quantity of a dark green, amorphous solid of low melting point, which could not be distilled. The methyl-alcoholic filtrate was then poured into water, the mixture extracted with ether, and the ethereal liquid deprived of a considerable amount of chlorophyll and a little unesterified acid by shaking with aqueous alkali, and subsequently washing with water. The ethereal solution was then evaporated, and the residue, which represented the crude methyl esters of the free fatty acids, purified by distillation under diminished pressure. The esters, which amounted to about 15 grams, were thus obtained as a pale brown oil, which was examined in connexion with the corresponding product from the combined acids, as described below.

Examination of the Fatty Acids.

The ethereal solutions of the neutral portion of the petroleum extract of the resin were united, and the solvent removed. The residue was then dissolved in alcohol, and hydrolysed by means of potassium hydroxide. After removing the greater part of the solvent, the mixture was poured into water, and then extracted many times with ether, for the removal of the unsaponifiable constituents. The alkaline aqueous liquid was then acidified, and extracted with ether, when a quantity of a sparingly soluble, dark-coloured solid separated in the lower portion of the ethereal layer. This solid was collected, and, when submitted to a process of purification analogous to that employed for the isolation of the previously-described phytosterolin, it yielded a further quantity of the latter. The ethereal filtrate was concentrated to a small bulk, and then largely diluted with petroleum, when a considerable amount of chlorophyll was precipitated, which was discarded. The ether-

petroleum liquid was then evaporated, and the residue dissolved in methyl alcohol, and esterified by means of sulphuric acid. On cooling the mixture a quantity of a dark green, wax-like solid separated, which was collected. This solid, on examination, was found to consist of chlorophyll, together with myricyl alcohol, which had escaped extraction during the removal of the unsaponifiable material. The methyl-alcoholic filtrate was poured into water, and the mixture extracted with ether, the resulting ethereal solution being deprived of some unesterified acid and much chlorophyll by extracting it with aqueous potassium hydroxide, and subsequently washing it with water. The ethereal liquid was then evaporated, and the residue purified by distillation under diminished pressure, when a quantity (about 15 grams) of a pale brown liquid was obtained. This portion of methyl ester, together with that previously obtained from the free fatty acids, was then fractionally distilled five times under diminished pressure, when the following fractions were obtained: Below 240°; 240—250°; 250—260°; above 260°/60 mm.

The fraction boiling below 240° represented about one-third of the total material, and consisted of methyl palmitate (m. p. 29°). On hydrolysis it yielded palmitic acid, which formed colourless plates melting at 62·5°. (Found, C=74·8; H=12·5. Calc., C=75·0; H=12·5 per cent.)

The fraction boiling at 240—250°/60 mm. was somewhat larger than the preceding one. On hydrolysis it yielded a mixture, which contained a small proportion of unsaturated acid, but consisted for the most part of palmitic acid, together with apparently a little stearic acid.

The portion of the material distilling at 250—260°/60 mm., when hydrolysed, gave a small amount of unsaturated acid, together with a solid acid. The latter, when crystallised five times alternately from alcohol and ethyl acetate, yielded stearic acid melting at 69°. (Found, C=75·9; H=12·7. Calc., C=76·1; H=12·7 per cent.)

The fraction of ester boiling above 260°/60 mm. was too small for examination.

Examination of the Unsaponifiable Material.

The combined ethereal extracts containing the unsaponifiable material, which had been obtained as above described, were washed, dried, and evaporated. The residue was then heated with a large amount of alcohol, and the mixture filtered whilst hot, thereby removing a quantity of almost black, tarry material. The filtrate, which was dark brown, deposited, on cooling, a quantity of an

indistinctly crystalline solid, which was collected, and again crystallised from alcohol. This was then crystallised once from ethyl acetate, and distilled under diminished pressure, when it passed over at a high temperature as an almost colourless liquid, which solidified on cooling. The distillate was then crystallised twice from ethyl acetate, with the employment of animal charcoal, when a quantity (about 8 grams) of small, colourless leaflets, melting at 83°, was obtained. (Found, C=82·1; H=14·5. $C_{30}H_{62}O$ requires C=82·2; H=14·5 per cent.)

This substance was therefore myricyl alcohol.

Isolation of a Phytosterol, $C_{27}H_{46}O$.

The combined alcoholic mother liquors from the crude myricyl alcohol were diluted somewhat with water, and kept for some time, when a quantity of crystalline material, together with much orange-red-coloured oil, was deposited. The solid was collected, when it was found to be a mixture of myricyl alcohol, and a substance which appeared to be a phytosterol. The myricyl alcohol was eliminated by fractional crystallisation from warm ethyl acetate, in which it was more sparingly soluble than the phytosterol. A product was then finally obtained, which, when crystallised from a mixture of ethyl acetate and dilute alcohol, formed large, lustrous plates, melting at 142—143°:

0·2932, on heating at 130°, lost 0·0162 H_2O. H_2O=5·5.

0·0986 * gave 0·2967 CO_2 and 0·1033 H_2O. C=83·6; H=11·9.

$C_{27}H_{46}O,H_2O$ requires H_2O=4·5 per cent.

$C_{27}H_{46}O$ requires C=83·9; H=11·9 per cent.

The optical rotation of the phytosterol was determined, with the following result:

0·2770,* made up to 20 c.c. with chloroform, gave α_D −1°3′ in a 2-dcm. tube, whence $[\alpha]_D$ −37·8°.

This phytosterol yielded an acetyl derivative, which formed pearly leaflets, melting at 128°.

The filtrate from the crude phytosterol, which contained the greater part of the unsaponifiable material, was carefully examined, but only orange-coloured, oily products could be obtained from it.

Ether, Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

The ether extract of the resin was a dark, brownish-green mass, and amounted to 14·4 grams. On examination it was found to contain, in addition to chlorophyll, some fatty matter, myricyl

* Dried at 130°.

alcohol, and a considerable proportion of rhein and aloe-emodin, but no kaempferol was present.

The chloroform extract of the resin amounted to only 3 grams, and consisted of a dark greenish-brown resin.

The ethyl acetate extract amounted to 2 grams, and was similar in character to the last-mentioned extract.

The alcohol extract was a dark brown resin, amounting to 20 grams, and nothing definite could be obtained from it.

II. *Senna Leaves from Peru.*

This material consisted of a sample of senna leaves which had been obtained from Lima, Peru, and concerning which the following information was supplied: "During the last few years there has been introduced into England, from Port Royal (Jamaica), and recently from the interior of Peru, a new species of senna (*Cassia lanceolata*, var. *Porturegalis*), the characters of which are as follows: The flavour of its infusion, which is clear, and nearly colourless, much resembles that of tea. It is very purgative, and at the same time it is not nauseous, nor does it cause griping or irritation. On account of these properties it is much valued for the debilitated, the aged, women, and children."

A sample of these leaves, together with buds, flowers, and pods, was submitted to Mr. E. M. Holmes, F.L.S., who kindly compared them with specimens at Kew and at the British Museum, when they were found to be, botanically, quite identical with well-developed specimens of Tinnevelly senna (*Cassia angustifolia*, Vahl). As is shown below, they differed somewhat from the Tinnevelly senna leaves, the examination of which has just been described, both in the amount of resin, the proportions of the various extracts, and in some of their constituents. The differences observed, however, are only such as might be accounted for by the altered conditions of climate and soil.

As a preliminary experiment, a portion (20 grams) of the ground leaves was extracted successively in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained:

Petroleum (b. p. 35—50°) extracted	0·82 gram	=	4·10	per cent.
Ether	,, 0·70 ,,		3·50	,,
Chloroform	,, 0·26 ,,		1·30	,,
Ethyl acetate	,, 0·47 ,,		2·35	,,
Alcohol	,, 2·25 ,,		11·25	,,
	Total = 4·50 grams	=	22·50	per cent.

A quantity (3·365 kilograms) of the ground leaves was then thoroughly extracted in a large Soxhlet apparatus with boiling

alcohol, after which the resulting extract was concentrated, and examined in a manner analogous to that described in connexion with the investigation of the Tinnevelly leaves.

A small amount of essential oil was removed by means of steam, after which the resin was separated from the water-soluble constituents. The aqueous liquid was extracted first with ether, and subsequently with amyl alcohol, after which it was found to contain considerable sugar, together with amorphous products, which yielded kaempferol on hydrolysis.

The ethereal extract, on examination, yielded, in addition to some amorphous products, aloe-emodin, rhein, and a product having the properties of a flavone derivative. The latter was, however, in part, much more sparingly soluble in alcohol than the corresponding material from the Tinnevelly leaves. The portion which was sparingly soluble crystallised from alcohol in small, yellow needles, melting at 302°. (Found, C = 60·9; H = 4·1. $C_{16}H_{12}O_7$ requires C = 61·2; H = 3·8 per cent.)

This substance was found to be identical with *iso*rhamnetin, which was first isolated by A. G. Perkin (T., 1896, **69**, 1658) from the flowers of the yellow wallflower, and subsequently by Power and Salway from red clover flowers (T., 1910, **97**, 245). The melting point observed in the present instance is, however, a little higher than that previously recorded. The substance yielded tetra-acetyl-*iso*rhamnetin, which formed thin, colourless needles, melting at 201°.

The alcoholic mother liquors from the *iso*rhamnetin gave a product which, when acetylated, yielded tetra-acetylkaempferol, identical with that obtained from the Tinnevelly leaves. It crystallised from alcohol with half a molecule of solvent of crystallisation:

0·1421,* on heating at 130°, lost 0·0062 C_2H_6O. $C_2H_6O = 4·6$.

$C_{23}H_{18}O_{10},\frac{1}{2}C_2H_6O$ requires $C_2H_6O = 4·8$ per cent.

The amyl alcohol extract of the aqueous liquid yielded some viscid, brown material, which was readily soluble in amyl alcohol, and a yellow, amorphous product, sparingly soluble in dry amyl alcohol. The viscid material, on treatment with alkali, yielded *iso*rhamnetin and kaempferol, the latter preponderating, whilst the sparingly soluble, yellow product was found to be a mixture of glucosides. It would not crystallise, and no individual substance could be separated from it. On hydrolysis with dilute aqueous sulphuric acid it yielded dextrose, rhein, *iso*rhamnetin, kaempferol, and aloe-emodin.

* Air-dried substance.

Examination of the Resin.

The resin was a soft, dark green mass, and amounted to 196 grams, being thus equivalent to 5·8 per cent. of the weight of the leaves employed.

The petroleum extract, which amounted to 137 grams, yielded, in addition to chlorophyll and amorphous products, the following definite substances: Myricyl alcohol (m. p. 83°); a phytosterol, $C_{27}H_{46}O$ (m. p. 142—143°); a phytosterolin, $C_{33}H_{56}O_6$; rhein; aloe-emodin; palmitic and stearic acids, and a small amount of unsaturated acid. Chrysophanol was absent.

The ether extract amounted to 18·5 grams. It consisted chiefly of resinous material and chlorophyll, but also yielded small amounts of rhein, aloe-emodin, and the above-mentioned mixture of flavone derivatives.

The chloroform, ethyl acetate, and alcohol extracts of the resin amounted to 9, 7, and 24 grams respectively. They all consisted of black, amorphous masses, and nothing definite could be isolated from any of them.

III. *Alexandrian Senna Leaves.*

In order to make the present investigation complete, it was deemed desirable also to make an examination of Alexandrian senna leaves, especially on account of the fact that Tschirch and Hiepe (*loc. cit.*) have stated that senna leaves contain "sennaemodin," which was considered to be identical with aloe-emodin, "senna-*iso*emodin," "sennarhamnetin," and "sennachrysophanic acid" (chrysophanol). In the present case no attempt was made to conduct a complete examination of the leaves, attention being chiefly directed to the examination of the anthraquinone and flavone derivatives, and their glucosides.

Ten pounds of a good quality of Alexandrian senna leaves were ground, and extracted in a large Soxhlet apparatus, first with ether, and then with alcohol, the resulting extracts being subsequently examined in the direction indicated, with the employment of methods similar to those already described. It was then found that the anthraquinone derivatives present consisted solely of rhein and aloe-emodin, whilst the flavone derivatives were *iso*rhamnetin and kæmpferol, the former preponderating. These four substances were also found to be present in the form of glucosides, and in much greater proportion as such than in the free state. Myricyl alcohol and a phytosterolin were also isolated.

Summary.

The results of the present investigations may be summarised as follows:

The material employed consisted of (I) Tinnevelly senna leaves (*Cassia angustifolia*, Vahl); (II) senna leaves from Lima, Peru, which were found to be botanically identical with the Tinnevelly leaves; (III) Alexandrian senna leaves. The last-mentioned species is usually recognised as *Cassia acutifolia*, Delile, but by some authorities it is regarded simply as a variety of *Cassia angustifolia*.

(I) An alcoholic extract of the Tinnevelly leaves, when distilled with steam, yielded a small amount of an essential oil. From the portion of the extract which was soluble in water the following substances were isolated: (i) Salicylic acid, (ii) rhein, $C_{15}H_8O_6$; (iii) kaempferol, $C_{15}H_{10}O_6$; (iv) aloe-emodin, $C_{15}H_{10}O_5$; (v) *kaempferin*, $C_{27}H_{30}O_{16},6H_2O$ (m. p. 185—195°), a new glucoside of kaempferol; (vi) a mixture of the glucosides of rhein and aloe-emodin; (vii) the magnesium salt of an unidentified organic acid. The aqueous liquid furthermore contained a quantity of a sugar which yielded *d*-phenylglucosazone (m. p. 216°), and some brown, amorphous products, which, on treatment with alkali, gave kaempferol, together with small amounts of rhein and aloe-emodin. Some amorphous, glucosidic material was also present.

The portion of the alcoholic extract which was insoluble in water consisted of a soft, dark green resin, which amounted to 7·0 per cent. of the weight of the leaves employed. From this material, which contained considerable chlorophyll and amorphous products, there were isolated, in addition to some of the substances mentioned above, the following compounds: (i) Myricyl alcohol; (ii) a phytosterol, $C_{27}H_{46}O$; (iii) a phytosterolin, $C_{33}H_{56}O_6$; (iv) palmitic and stearic acids.

(II) The senna leaves from Lima, Peru, were found to contain all the above-mentioned compounds, with the exception of the magnesium salt, and, in addition, *iso*rhamnetin. A glucoside of *iso*rhamnetin was also present in association with glucosides of kaempferol, rhein, and aloe-emodin, but no pure compound could be isolated from the mixture.

(III) Alexandrian senna leaves yielded, in addition to myricyl alcohol and a phytosterolin, rhein, aloe-emodin, kaempferol, and *iso*rhamnetin. These four substances were also present in the form of glucosides, and in much greater proportion as such than in the free state.

The statements of Tschirch and Hiepe (*Arch. Pharm.*, 1900, **238**, 427) that senna leaves contain "senna*iso*emodin," "senna-

chrysophanic acid" (chrysophanol), and a "substance, $C_{14}H_{10}O_5$," could not be confirmed, it having been ascertained that the anthraquinone derivatives present consist solely of rhein and aloe-emodin. In this connexion it may be noted that a mixture of approximately equal quantities of the last-mentioned two compounds has the empirical composition and properties assigned by Tschirch and Hiepe to the "substance, $C_{14}H_{10}O_5$." Furthermore, the "sennarhamnetin" of the last-mentioned authors has been found to be identical with the *iso*rhamnetin previously described by Perkin (T., 1896, **69**, 1658).

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